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PHENOLIC COMPOUNDS FROM THE RHIZOMES OF *ALPINIA SPECIOSA*

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Key Word Index—*Alpinia speciosa*; Zingiberaceae; dihydroflavokawin B; flavokawin B; biogenesis; ^{13}C NMR; dihydrochalcone; chemotaxonomy.

Abstract—From the rhizomes of *Alpinia speciosa*, a new dihydrochalcone, dihydroflavokawin B, has been isolated together with six known phenolic compounds. The chemotaxonomic significance of these findings is discussed briefly.

INTRODUCTION

During the biological evaluation of crude drugs and plant materials for the presence of active substances, it has been found that methanolic extracts from the rhizomes of *Alpina speciosa* possess significantly inhibitory activities against histamine and barium chloride by the Magnus method using excised guinea pig ileum. This observation led us to investigate the constituents of the rhizomes of this plant, and some known sesquiterpenes have already been isolated as the active substances [1] along with two new diterpenes, labda-8(17),12-diene-15,16-dial and 15,16-bisnorlabda-8(17),11-dien-13-one [2].

In this paper, we report the isolation and characterization of one new and six known phenolic compounds and discuss the chemotaxonomic usefulness of these compounds.

RESULTS

The aqueous methanol extracts of the fresh rhizomes of *A. speciosa* were shaken with petrol and then with chloroform. The petrol-soluble fraction was repeatedly separated by Si gel chromatography to give three compounds (**1**, **2** and **3**).

Compound **1** possessing sweet odor was readily identified as methyl *trans*-cinnamate, which has frequently been isolated from zingiberaceous plants. Compound **3** revealed a strong UV absorption maximum at 342 nm ($\varepsilon = 27000$) characteristic of chalcone. The IR and NMR spectra agreed almost completely with those of flavokawin B, which has previously been isolated from *Piper methysticum* [3]. The identity was established by direct comparison with the authentic specimen. We also have found the same compound to occur in the rhizomes of *A. japonica* (unpublished results).

Compound **2**, obtained as colorless needles, mp 106.0–106.5°, $C_{17}H_{18}O_4$, showed UV maxima at 217 nm (log $\varepsilon = 4.16$, infl.), 288 nm (4.28) and 328 nm (3.53, infl.); on addition of alkali, a bathochromic shift was observed, suggesting the presence of a hydroxyketone moiety. The IR absorption of this ketone moiety appeared at 1630 cm^{-1} due to conjugated chelation. The ^1H NMR spectrum of **2** revealed two adjacent methylene signals at δ 2.94 (2 H, *t*, $J = 8$ Hz) and 3.08 (2 H, *t*, $J = 8$ Hz), and the other signals assigned to two methoxyl groups and seven aromatic protons were quite similar to those of flavokawin B (**3**). Taking into account the presence of **3** in the same plant, **2** was assumed to be dihydroflavokawin B. The structure was finally established by direct comparison with a sample produced from **3** by catalytic hydrogenation.

From the chloroform-soluble fraction, four compounds (**4**, **5**, **6** and **7**) were isolated. The major components, **4** and **5**, of this fraction were identified by comparison with authentic speciments of dihydro-5,6-dehydrokawain and 5,6-dehydrokawain, respectively, which have previously been isolated from the same plant by Kimura *et al.* [4]. Compounds **6** and **7** were identified as cardamomin and alpinetin, respectively; the occurrence of these two compounds (**6** and **7**) in the seeds of the plant has been reported [5].

The ^{13}C NMR signals of compounds **2**–**7** were assigned by substituent chemical shift and by comparison with those of the analogous compounds (Table 1).

DISCUSSION

From the biogenetic standpoint, it is noteworthy that the phenolics now isolated from the rhizomes of *A. speciosa* possess an unsubstituted phenyl group in their structures. Moreover, the same partial structure is found in the phenolics isolated from the rhizomes of other *Alpinia* species, namely, 1-(4′-hydroxy-3′-methoxyphenyl)-7-phenyl-3-heptanone of *A. oxyphylla* [6], 5-hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone of *A. officinarum* [7], alpinon of *A. japonica* [8], izalpinin of *A. japonica* [8] and *A. intermedia* [9], galangin of *A. galanga* [10] and cardamomin of *A. katsumadai* [11]. There are only two exceptions: kaempferol and kumatakenin in *A. kumatake* [12]. Therefore, this structural feature seems to be characteristic of the rhizome phenolic constituents in the genus *Alpinia*.

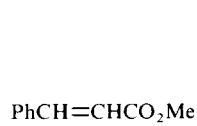
EXPERIMENTAL

Mps are uncorrected. For the NMR data, chemical shifts are expressed in δ ppm from tetramethylsilane as in internal standard and coupling constants (J) are given in Hz. Si gel (Merck 70–230 mesh) was used for CC and Kieselgel 60 F_{254}, 0.25 mm, was used

Table 1. ^{13}C NMR (δ ppm) spectral data of *Alpinia* phenolics*

Carbon assignment	2	3	4	5	6	7
1	141.7 *s*	135.5 *s*	—	—	136.5 *s*	—
2	128.3 *d*	128.3 *d*	164.2 *s*[b]	158.5 *s*	129.0 *d*	78.1 *d*
3	128.3 *d*	128.7 *d*	87.6 *d*	88.8 *d*	129.7 *d*	45.0 *t*
4	125.9 *d*	130.0 *d*	171.0 *s*	171.0 *s*	130.7 *d*	187.4 *s*
5	128.3 *d*	128.7 *d*	100.1 *d*	101.3 *d*	129.7 *d*	164.1 *s*
6	128.3 *d*	128.3 *d*	164.7 *s*[b]	163.9 *s*	129.0 *d*	95.8 *d*
7	30.7 *t*	127.5 *d*	35.8 *t*	118.5 *d*	128.6 *d*	164.4 *s*
8	45.7 *t*	142.2 *d*	32.8 *t*	135.6 *d*	142.4 *d*	93.5 *d*
9	204.3 *s*	192.5 *s*	139.8 *s*	135.1 *s*	193.0 *s*	162.2 *s*
10	—	—	128.2 *d*	127.4 *d*	—	104.6 *s*
11	—	—	128.5 *d*	128.8 *d*	—	—
12	—	—	126.3 *d*	129.3 *d*	—	—
13	—	—	128.5 *d*	128.8 *d*	—	—
14	—	—	128.2 *d*	127.4 *d*	—	—
1′	105.7 *s*	106.3 *s*	—	—	106.4 *s*	139.2 *s*
2′	165.9 *s*[a]	166.1 *s*	—	—	168.3 *s*	126.4 *d*
3′	90.8 *d*	91.2 *d*	—	—	92.3 *d*	128.5 *d*
4′	167.6 *s*[a]	168.3 *d*	—	—	165.8 *s*	128.3 *d*
5′	93.0 *d*	93.8 *d*	—	—	97.0 *d*	128.5 *d*
6′	162.6 *s*	162.4 *s*	—	—	164.3 *s*	126.4 *d*
4-OMe	—	—	55.8 *q*	55.9 *q*	—	—
7-OMe	—	—	—	—	—	55.7 *q*
2′-OMe	55.5 *q*	55.5 *q*	—	—	56.3 *q*	—
4′-OMe	55.5 *q*	55.5 *q*	—	—	—	—

* All data were obtained in $CDCl_3$ except **3** (Me_2CO-d_6) and **7** (DMSO-d_6).
Signals bearing the same alphabetical superscript in any one column may be reversed.



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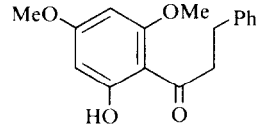

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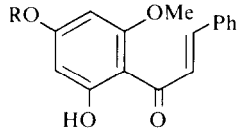

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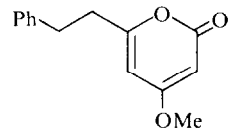
6 R = H



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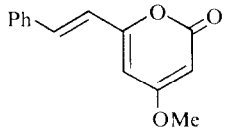

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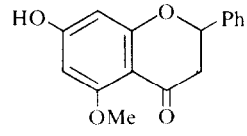

7

for TLC. Detection was by spraying with 10% H_2SO_4 and heating. Prep. HPLC used CIG column system 22ϕ × 300 mm (Kusano Scientific Co., Tokyo) and stationary phase used Iatrobeads (60 μ spherical Si gel, Iatron Co., Tokyo).

Extraction of Alpinia speciosa. The fresh rhizomes (87.5 kg) of *A. speciosa* collected in Miyake Island, Tokyo, early in September 1978 were crushed and extracted with MeOH (×3) at room temp. The MeOH extracts were diluted with water to about 10% aq. MeOH and these were extrd with petrol. The petrol layer was evapd to give a brown oil (94.9 g).

The aq. MeOH layer was concd under red. pres. to about 1/8 volume and extrd with $CHCl_3$. Evaporation of the $CHCl_3$ left a semi-crystalline mass (266.9 g). The aq. MeOH layer was further concd to remove the MeOH and extrd with EtOAc. The EtOAc layer and the residual layer were evapd to dryness to afford a brown resin (88.2 g) and a dark brown tar (1956.3 g), respectively.

Isolation of **1–3**. A portion of the petrol-soluble fraction (4.83 g) was chromatographed over a column of Si gel. The column was eluted with *n*-hexane–Et_2O (9:1) to give an oil (0.23 g), which on repeated CC yielded **1** (150 mg). Another portion of the petrol fraction (35 g) was also chromatographed over a column of Si gel (100 g) packed in *n*-hexane. The column was eluted with *n*-hexane containing increasing amounts of Et_2O. Elution with *n*-hexane–Et_2O (17:3) gave a brown oil (0.44 g), which was separated by prep. HPLC (C_6H_6). The initial fractions, yielding crystals on evaporation, were purified by recrystallization from *n*-hexane to afford **2** (41 mg). Further elution with *n*-hexane–Et_2O (4:1) gave a brown heavy oil (1.0 g) showing a yellow spot on TLC. Half of the oil was subjected to prep. HPLC (*n*-hexane–EtOAc, 9:1). The yellow crystals (102.5 mg) thus obtained were recrystallized from *n*-hexane to afford **3** (37 mg).

Isolation of **4–7**. The $CHCl_3$ fraction (25.2 g) was chromatographed over a column of Si gel (1.25 kg), and eluted first with $CHCl_3$ and then increasing amounts of MeOH in $CHCl_3$. Elution with $CHCl_3$ gave a large quantity of yellowish crystals. Repeated recrystallization from Et_2O led to the isolation of **4** (5.1 g). The mother liquors were combined and evapd to dryness, and the residue was recrystallized from EtOH to give **5** (3.4 g).

The fractions eluted with $CHCl_3$–MeOH (49:1) were combined and evapd to give a brown oil (1.44 g). After treatment with *n*-hexane–EtOAc (1:1), a small amount of crystals that separated was filtered. The crystals were recrystallized from MeOH to yield **7** (40 mg). The filtrate was passed through a prep. HPLC column using *n*-hexane–EtOAc (1:1) as the eluant. The fractions showing a yellow spot on TLC were collected and worked up. Recrystallization from MeOH gave **6** (30 mg).

1 (*methyl* trans-*cinnamate*). Colourless needles, mp 32.0–33.0°, $C_{10}H_{10}O_2$, MS 70 eV *m/z* (rel. int.) 162 [M^+] (44), 131 (100), 103 (57), 77 (39). IR ν_{max}^{KBr} cm^{-1}: 1720, 1638, 1170, 985, 773. UV λ_{max}^{EtOH} nm (log ε): 266 (4.33). ^1H NMR δ ppm in $CDCl_3$: 3.78 (3 H, *s*, MeO–), 6.20 (1 H, *d*, J = 16 Hz, 2-H), 7.20–7.60 (5 H, *m*, aromatic), 7.62 (1 H, *d*, J = 16 Hz, 3-H).

2 (*dihydroflavokawin B*). Colourless needles, mp 106.0–106.5°, $C_{17}H_{18}O_4$, High MS: Calc. for 286.120, found; 286.121. (Found: C, 71.54; H, 6.38. $C_{17}H_{18}O_4$ requires: C, 71.31; H, 6.34%.) MS 70 eV *m/z* (rel. int.): 286 [M^+] (26), 181 (100), 154 (31), 91 (10). IR ν_{max}^{KBr} cm^{-1}: 1630, 1596, 1430, 1170, 760. UV λ_{max}^{EtOH} nm (log ε): 214 (infl. 4.37), 288 (4.28). $\lambda_{max}^{EtOH+NaOH}$ nm: 210, 234 (infl.), 288, 330 (infl.). ^1H NMR δ ppm in $CDCl_3$: 2.94 (2 H, *t*, J = 7 Hz), 3.28 (2 H, *t*, J = 7 Hz, 8-H), 3.76 (6 H, *s*, MeO- ×2), 5.88 (1 H, *d*, J = 3 Hz, 4′-H), 6.04 (1 H, *d*, J = 3 Hz, 5′-H), 7.20 (5 H, *s*, aromatic), 14.00 (1 H, *s*, disappeared with D_2O). ^{13}C NMR spectrum is given in Table 1.

3 (*flavokawin B*). Yellow needles, mp 91.5–92.0°, $C_{17}H_{16}O_4$, MS 70 eV *m/z* (rel. int.): 284 [M^+] (64), 207 (100), 181 (32). IR ν_{max}^{KBr} cm^{-1}: 2950, 1630, 1590, 1445, 1350, 1220, 820, 745. UV λ_{max}^{EtOH} nm (log ε): 342 (4.43). ^1H NMR δ ppm in $CDCl_3$: 3.89, 3.88 (each 3 H, *s*, MeO–), 5.96 (1 H, *d*, J = 3 Hz, 5′-H), 6.10 (1 H, *d*, J = 3 Hz, 3′-H), 7.32–7.90 (5 H, *m*, aromatic), 14.28 (1 H, *s*, disappeared with D_2O). ^{13}C NMR spectrum is in Table 1.

4 (*dihydro-5,6-dehydrokawain*). Colourless needles, mp 96.0–97.0°, $C_{14}H_{14}O_3$, MS 70 eV *m/z* (rel. int.): 230 [M^+] (100), 202 (18), 125 (97), 91 (97), 69 (48). IR ν_{max}^{KBr} cm^{-1}: 1730, 1700, 1650, 1570, 1500, 1240, 745. UV λ_{max}^{EtOH} nm (log ε): 277 (3.86). ^1H NMR δ ppm in $CDCl_3$: 2.68 (2 H, *t*, J = 8 Hz, CH_2), 2.96 (2 H, *t*, J = 8 Hz, CH_2), 3.72 (3 H, *s*, MeO–), 5.32 (1 H, *d*, J = 2 Hz, 3-H), 5.70 (1 H, *d*, J = 2 Hz, 5-H), 6.98–7.32 (5 H, *m*, aromatic). ^{13}C NMR spectrum in Table 1.

5 (5,6-*dehydrokawain*). Pale yellow needles, mp 136.5–138.5°, $C_{14}H_{12}O_3$, MS 70 eV *m/z* (rel. int.): 288 [M^+] (100), 200 (40), 157 (27), 77 (19), 69 (17). IR ν_{max}^{KBr} cm^{-1}: 3040, 1740, 1610, 705. UV λ_{max}^{EtOH} nm (log ε): 220 (4.20), 228 (4.20), 252 (4.13), 342 (4.39). ^1H NMR δ ppm in $CDCl_3$: 3.78 (3 H, *s*, MeO–), 5.46 (1 H, *d*, J = 2 Hz, 3-H), 5.94 (1 H, *d*, J = 2 Hz, 5-H), 6.48 (1 H, *d*, J = 16 Hz, 7-H), 7.46 (1 H, *d*, J = 16 Hz, 8-H), 7.30 (5 H, *m*, aromatic). ^{13}C NMR spectrum in Table 1.

6 (*cardamomin*). Yellow needles, mp 195.0–196.0°, $C_{16}H_{14}O_4$, MS 70 eV *m/z* (rel. int.): 270 [M^+] (66), 269 (51), 253 (9), 193 (100), 131 (9), 103 (20), 77 (19). IR λ_{max}^{KBr} cm^{-1}: 3140, 1630, 1495, 1340, 1220, 1180, 1120, 980, 795, 750. UV λ_{max}^{EtOH} nm (log ε): 344 (4.35), 320 (infl. 4.28), 212 (4.42). $\lambda_{max}^{EtOH+NaOH}$ nm: 390, 296, 268, 212. ^1H NMR δ ppm in $(CD_3)_2CO$: 3.98 (3 H, *s*, MeO–), 6.02 (1 H, *d*, J = 3 Hz), 6.08 (1 H, *d*, J = 3 Hz), 7.36–7.76 (5 H, *m*), 7.80 (1 H, *d*, J = 16 Hz, α-position), 7.94 (1 H, *d*, J = 16 Hz, β-position), 14.14 (1 H, *s*, disappeared with D_2O). ^{13}C NMR spectrum in Table 1.

7 (*alpinetin*). Colourless needles, mp 222–223°, $C_{16}H_{15}O_4$ (Found: C, 70.91; H, 5.16, Calc. for $C_{14}H_{14}O_4$: C, 71.10; H, 5.22.) MS 70 eV *m/z* (rel. int.): 270 [M^+] (58), 255 (2), 242 (2), 193 (20), 166 (100), 138 (29), 104 (13). UV λ_{max}^{EtOH} nm (log ε): 320 (4.00), 286 (4.14), 209 (4.42). $\lambda_{max}^{EtOH+NaOH}$ nm: 328, 250, 212. ^1H NMR δ ppm in DMSO-d_6: 2.59 (1 H, *d.d*, J = 14 Hz, AB part ABX system, 3-H), 2.98 (1 H, *d.d*, J = 14 Hz, 12 Hz, AB part ABX system, 3-H), 3.72 (3 H, *s*), 5.44 (1 H, *d.d*, J = 12 Hz, 4 Hz, X part ABX system, 2-H), 5.98 (1 H, *d*, J = 2 Hz, 6-H), 6.06 (1 H, *d*, J = 2 Hz, 8-H), 7.40 (5 H, *m*). ^{13}C NMR spectrum in Table 1.

Catalytic hydrogenation of flavokawin B. To a suspension of Pd/C (32 mg, satd with hydrogen) in EtOH (5 mg) was added a soln of flavokawin B (27.6 mg) in EtOH (5 ml). The mixture was stirred vigorously and allowed to absorb hydrogen at atm pres. for 48 hr. After filtration, the solvent was removed to give colourless crystals which were recrystallized from *n*-hexane.

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